EPHX1 Polymorphisms and the Risk of Lung Cancer
A HuGE Review

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Background: Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both the activation and detoxification of tobacco-derived carcinogens. Polymorphisms at exons 3 and 4 of the *EPHX1* gene have been reported to be associated with variations in EPHX1 activity. The aim of this study is to review and summarize the available molecular epidemiologic studies of lung cancer and EPHX1.

Methods: We searched MEDLINE, Current Contents, and Web of Science databases for studies published before August 2004. We conducted a systematic review and meta-analysis of 13 case–control studies. Summary odds ratios and summary prevalence of the variant allele (genotype) of both polymorphisms in the *EPHX1* gene were calculated using the DerSimonian and Laird method.

Results: The low-activity (variant) genotype of *EPHX1* polymorphism at exon 3 was associated with decreased risk of lung cancer (odds ratio = 0.65; 95% confidence interval = 0.44–0.96) in lung cancer risk among whites. In white populations, the high-activity (variant) genotype of *EPHX1* polymorphism at exon 4 was associated with a modest increase in risk of lung cancer (1.22; 0.79–1.90) and the predicted low activity was associated with a modest decrease in risk (0.72; 0.43–1.22).

Conclusions: EPHX1 enzyme may act as a phase I enzyme in lung carcinogenesis. The low-activity genotype of *EPHX1* gene is associated with decreased risk of lung cancer among whites.

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Polycyclic aromatic hydrocarbons and aromatic amines are classes of compounds known to produce human cancers. There has been much attention to the role of genetic variability in the metabolism of these compounds and the effects on human susceptibility. In this article, we review the literature on variants of one such gene, microsomal epoxide hydrolase 1, and their association with lung cancer susceptibility.

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GENES

Microsomal Epoxide Hydrolase 1

Microsomal epoxide hydrolase 1 (EPHX1, EC 3.3.2.3) plays an important role in both the activation and detoxification of polycyclic aromatic hydrocarbons and aromatic amines. This enzyme is a protective enzyme involved in general oxidative defenses against a number of environmental substances, whereas it is also involved in the xenobiotic activation of carcinogens.[1–3] EPHX1 catalyzes the hydrolysis of arene, alkene, and aliphatic epoxides from polycyclic aromatic hydrocarbons and aromatic amines. This hydrolysis is generally a detoxification reaction because less reactive and more water-soluble *trans*-dihydrodiols are produced.[3] However, in the case of some hydrocarbons such as benzo(a)pyrene (BP) present in tobacco smoke, more highly reactive and mutagenic compounds, for example benzo(a)pyrene 7,8-diol-9,10 epoxide, are generated in the metabolic process.[4] EPHX1 activity has been detected in all tissues (microsome, endoplasmic reticulum, and integral to membrane), and the highest concentrations have been found in lung, liver, kidney, gonads, and epithelial cells.[5–7] The activation or inactivation effects of EPHX1 may depend on the specific compounds being metabolized.

VARIANTS

The *EPHX1* gene, also known as *MEH*, *EPHX*, *EPOX*, or faklor, consists of 9 exons and 8 introns on chromosome 1q42.1.[8] It covers 35.48 kb, from 222972424 to 223007900, on the direct strand. In the coding region of the *EPHX1* gene, 2 relatively common genetic polymorphisms are characterized within exons 3 and 4.[9,10] In exon 3 of the *EPHX1* gene, a C has been substituted for a T, resulting in a tyrosine replacement by histidine at codon 113 (Tyr113His). In vitro expression analyses indicate that this amino acid replacement results in a 40% to 50% decrease in enzyme activity. Another polymorphism occurs in exon 4, a C to A transition, causing a histidine to arginine change at codon 139 (His139Arg). This change results in a 25% increase of enzyme activity.[10]

Based on the assumption that the Tyr allele at exon 3 and the His allele at exon 4 confer normal activity, whereas the His allele at exon 3 confers low activity and the Arg allele at exon 4 confers high activity, Smith and Harrison[1] and

Benhamou et al[11] classified predicted EPHX1 activity as low, intermediate, or high on the presence or absence of the 2 polymorphisms (Table 1).

EPHX1 Try113His polymorphism frequencies in different populations are shown in Appendix Table 1 (available with online version of this article). The frequencies of the His (variant) allele at exon 3 in controls were most common among Asians (summary frequency based on random effects model = 51.2%; 95% CI = 46.2–56.2%) and least common among blacks (19.3%; 12.7–26.0%) with an intermediate frequency of 33.8% (30.6–37.0%) among whites. Summary frequencies of the Arg (variant) allele at exon 4 among Asians, whites, and blacks based on a random effects model were 13.8% (11.3–16.3%), 18.7% (17.5–19.8%), and 27.1% (24.0–30.3), respectively (Appendix Table 2). Clear ethnic differences were seen in both the polymorphisms. A T-4238A transversion in the 5′-flanking region was found as a heterozygous change in 19.0% and as a homozygous change in 1.5% of whites (n = 277).[23] A C2557G transversion in intron 1 was found as a heterozygous change in 16% and as a homozygous change in 1.6% in a population (not specified, n = 509).[56] However, no studies on lung cancer and these polymorphisms have been reported to date. The decrease in promoter activity resulting from the C2557G transversion and the T-4238A transversion was 86% and 53%, respectively.[56]

DISEASE

Although the incidence of lung cancer has peaked in the United States and most of Europe, lung cancer is showing increasing incidence and mortality in many countries around the world. The number of new cases of lung cancer diagnosed worldwide in 2000 was estimated to be 1,239,000 (902,000 men and 337,000 women), accounting for 12% of all new cases of cancer; 1,103,000 (810,000 men and 293,000 women) died of the disease, accounting for 18% of all deaths from cancer.[57] This disease ranks as the foremost cancer killer in men and the second largest in women. The case-fatality (ratio of mortality to incidence), which is an indicator of prognosis, is 0.89 for lung cancer (the third worst after cancer of the pancreas [0.99] and liver [0.97]).[58]

Genetic Epidemiology

Given that all smokers do not develop lung cancer, a genetic component for this cancer seems plausible. Cigarette smoke contains several thousand chemicals, of which approximately 50 compounds are known carcinogens. These include polycyclic aromatic hydrocarbons, aromatic amines, and N-nitroso compounds. Some of these compounds are reactive carcinogens, but most are procarcinogens that must be activated by phase I enzymes such as those encoded by the cytochrome P450 (*CYP*) multigene superfamily of mixed function mono-oxygenases and then converted into reactive carcinogens. All reactive carcinogens can bind to DNA and form DNA adducts that are capable of inducing mutations and initiating carcinogenesis. *CYPs* such as *CYP1A1*, *CYP1A2*, *CYP2A6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, and *CYP3A4* are primarily involved in the drug metabolism.[59] Other phase I enzymes, which may influence the risk of lung cancer, are MPO, EPHX1, NQO1, and alcohol dehydrogenase.

Following the phase I reaction, phase II enzymes such as glutathione S-transferases (GSTs) are responsible for detoxifying the activated forms of polycyclic aromatic hydrocarbons epoxides. The GSTs are constitutively found in a wide variety of tissues with different characteristic patterns of GST isozymes. Other phase II enzymes that may influence the risk of lung cancer are EPHX1, NQO1, N-acetyltransferases (NATs), UDP-glucuronosyltransferase, aldehyde dehydrogenase, sulfotransferase, and superoxide dismutase.

EPHX1 as well as *CYPs*, *GSTs*, and *NATs* may have a critical role in lung carcinogenesis, but the association of *EPHX1* and lung cancer risk has been less reviewed than the others (only 1 review in 2002).[60] In this review, we performed a metaanalysis of 11 published studies to obtain summary measures of the effects of exon 3 polymorphism, exon 4 polymorphism, and the predicted activity based on the presence or absence of 2 polymorphisms of *EPHX1* gene in the etiology of lung cancer.

TABLE 1. Predicted EPHX1 Activity*

His139Arg Polymorphism at Exon 4	Tyr113His Polymorphism at Exon 3		
	Try/Try	Try/His	His/His
His/His	Intermediate (Intermediate)	Low (low)	Low (Very low)
His/Arg	High (High)	Intermediate (Intermediate)	Low (Very low)
Arg/Arg	High (High)	High (Low)	Intermediate (Very low)

*Classification based on Benhamou et al.[11]
Classification based on Smith and Harrison[1] is in parentheses.

STATISTICAL METHODS

Identification and Eligibility of Relevant Studies

We conducted MEDLINE, Current Contents, and Web of Science searches using "microsomal epoxide hydrolase 1," "lung cancer," and "polymorphism" for papers published before August 2004. Additional articles were identified through the references cited in the first series of articles selected. Articles included in metaanalysis were in any language, with human subjects, published in the primary literature and with had no obvious overlap of subjects with other studies. We excluded studies with the same data or overlapping data by the same authors. Case–control studies were eligible if they had determined the distribution of the relevant genotypes in lung cancer cases and in concurrent controls using a molecular method for genotyping. Using the MEDLINE database, we identified 13 case–control studies that provided information on lung cancer occurrence associated with the *EPHX1* polymorphisms. One meeting abstract, identified through the Web of Science database, has been excluded because of poor availability. No additional articles through Current Contents have been identified. Details regarding the 13 included studies are shown in Appendix Table 3.

TABLE 2. Studies of *EPHX1* Try113His Polymorphism at Exon 3 and Risk of Lung Cancer

Citation	Race/Ethnicity	No. Cases*	No. Controls*	His/His Genotype		
				Cases	Controls	OR (95% CI)
Smith et al,[1] UK	White	50	203	10.0	6.4	1.6 (0.6–4.8)
Benhamou et al,[11] France	White	150	172	14.7	18.0	0.50 (0.26–1.04)
Persson et al,[42] Sweden	White	74	122	27.0	18.0	1.76 (0.80–3.86)
London et al,[15] USA	Black	155	242	0.6	5.0	0.08 (0.01–0.62)
	White	182	458	8.2	8.1	0.99 (0.46–2.14)
Yoshikawa et al,[43] Japan	Japanese	71	107	16.9	19.6	0.8 (0.4–1.8)
To-Figueras et al,[18] Spain	White	175	187	4.6	8.0	0.44 (0.27–0.71)
Yin et al,[44] China	Chinese	84	84	17.9	16.7	1.71 (0.65–4.54)
Wu et al,[54] USA	Mexican-American	51	64	9.8	10.9	1.0 (0.22–4.29)
	Black	65	62	4.6	6.4	0.71 (0.10–4.53)
Zhao et al,[20] USA	White	162	153	12.3	14.4	0.81 (0.39–1.70)
Cajas-Salazar et al,[13] USA	White	110	119	5.5	4.2	1.07 (0.25–4.59)
Gsur et al,[21] Austria	White	277	496	5.8	10.9	0.38 (0.20–0.75)

Summary	Hardy-Weinberg Equilibrium	No. Populations	No. Cases	No. Controls	His Allele % (95% CI)	His/His Allele % (95% CI)	OR (95% CI) for His/His Genotype (compared with Tyr/Tyr genotype)		Cochrane Q Test for Heterogeneity
							Random-Effects Model	Fixed-Effects Model	
All populations		13	1606	2469	32.4 (28.6–36.2)	10.4 (7.9–12.9)	0.83 (0.61–1.12)	0.81 (0.63–1.02)	0.12
White populations		7	1106	1788	31.4 (28.4–34.4)	9.5 (6.7–12.2)	0.71 (0.52–0.99)	0.70 (0.53–0.93)	0.27
White populations	$P_{HWE} \geq 0.05$	5	894	1432	31.5 (27.4–35.6)	9.4 (6.0–12.7)	0.65 (0.44–0.96)	0.64 (046–0.89)	0.23
Asian populations	$P_{HWE} \geq 0.05$	3	229	313	43.1 (37.6–48.6)	18.2 (13.9–22.4)	1.37 (0.83–2.27)	1.37 (0.83–2.27)	0.40

*Number of subjects genotyped.

TABLE 3. Studies of *EPHX1* His139Arg Polymorphism at Exon 4 and Risk of Lung Cancer

Citation	Race/Ethnicity	No. Cases*	No. Controls*	Arg/Arg Genotype		
				Cases; %	Controls; %	OR (95% CI)
Smith et al,[1] UK	White	50	203	2.0	1.5	1.4 (0.1–13.3)
Benhamou et al,[11] France	White	150	172	2.0	1.2	1.22 (0.72–2.04)†
Persson et al,[42] Sweden	Chinese	74	117	0	2.6	0.26 (0.08–2.34)
London et al,[15] USA	Black	155	242	9.7	7.4	1.05 (0.45–2.49)
	White	182	458	3.8	4.4	0.63 (0.23–1.77)
Yoshikawa et al,[43] Japan	Japanese	71	107	2.8	3.7	0.7 (0.1–4.2)
To-Figueras et al,[18] Spain	White	175	187	1.7	3.7	0.55 (0.33–0.91)
Yin et al,[44] China	Chinese	84	84	1.1	1.1	1.06 (0.07–17.34)
Wu et al,[54] USA	Mexican-American	56	73	5.4	1.4	5.0 (0.38–206.4)
	Black	75	71	8.0	1.4	6.6 (0.71–331.4)
Zhao et al,[20] USA	White	166	157	6.0	3.8	1.86 (0.59–6.46)
Cajas-Salazar et al,[13] USA	White	110	119	5.5	1.7	6.26 (1.02–38.3)
Gsur et al,[21] Austria	White	277	496	4.0	3.2	1.83 (0.76–4.44)

Summary	Hardy-Weinberg Equilibrium	No. Populations	No. Cases	No. Controls	Arg Allele % (95% CI)	Arg/Arg Genotype % (95% CI)	OR (95% CI) for Arg/Arg Genotype (compared with His/His genotype)		Cochrane Q Test for Heterogeneity
							Random-Effects Model	Fixed-Effects Model	
All populations		13	1625	2486	16.6 (13.5–19.7)	2.7 (1.8–3.5)	1.35 (0.94–1.92)	1.35 (0.94–1.92)	0.61
White populations	$P_{HWE} \geq 0.05$	7	1110	1792	17.5 (15.8–19.3)	2.6 (1.6–3.6)	1.22 (0.79–1.90)	1.22 (0.79–1.90)	0.50
Asian populations	$P_{HWE} \geq 0.05$	2	155	191	11.6 (7.3–16.0)	2.1 (0.5–3.7)	0.89 (0.20–3.90)	0.89 (0.20–3.90)	0.89

*Number of subjects genotyped.
†Arg/Arg and His/Arg genotypes combined.

Data Extraction and Assessment of Study Quality

For each study, 2 investigators (CK and KY) independently extracted the following characteristics: authors, year of publication, place of study, ethnic group of the study population, characteristics of lung cancer cases (age distribution, sex ratio, histologic type, smoking, and occupational exposure), characteristics of controls (age distribution, sex ratio, source of population, smoking, and occupational exposure), number of genotyped cases and controls, frequency of the genotypes, ORs, adjusted factors for OR, and the method for quality control of genotyping. In some cases, the OR or the 95% CI was not reported in the publication, but we could derive it from the raw data presented. For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group whenever possible.

Methods for defining study quality in genetic studies are more clearly defined than those for observational studies. We assessed the Hardy-Weinberg equilibrium (HWE) through a goodness-of-fit chi-squared test (Pearson) to compare the observed and expected genotype frequencies among controls. We also assessed the homogeneity of the study population (white only or mostly white).

Metanalysis

Data were combined using both fixed-effects (Mantel-Haenszel) and random-effect (DerSimonian and Laird method) models.[61] Random-effects model are more appropriate when heterogeneity is present.[61] Thus, estimates values were basically based on random-effects model. Heterogeneity, evaluated by the Cochrane Q test among the studies, was considered significant for $P < 0.10$.[62,63] To test for publication bias, both Begg's[64] and Egger's[65] tests were used to assess whether smaller studies reported greater associations than larger studies. Publication bias was considered significant for $P < 0.10$. In a sensitivity analysis (subgroup analysis), we combined only studies with allelic frequencies being in HWE (Pearson χ^2 test, $P \geq 0.05$) because departure from HWE can imply the presence of genotyping error, possible ethnic admixture in the population, or selection bias (lack of representativeness of the general population). All the calculations were performed with computer program STATA Version 8.2 (Stata Corp., College Station, TX).

GENOTYPING METHODS

Traditionally, genotyping for metabolic enzyme single nucleotide polymorphisms has been conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Recently, Taqman real-time PCR chemistry has been adapted for use in allelic discrimination assays. Generally, concordance rate between PCR-RFLP genotyping and the real-time PCR assay is considered to be high. As for cytokine genes, the Taqman real-time PCR assay is highly accurate with an error rate of <1% and concordance rate with PCR-RFLP genotyping of 99.4%.[66] Gsur et al[21] reassessed the *EPHX1* exon 3 genotypes using TaqMan-based real-time PCR because the PCR-RFLP method for the exon 3 polymorphism is potentially inaccurate due to another nearby polymorphism.[16] Gsur et al found over 50% of heterozygote subjects falsely classified as homozygotes.[21] Although PCR-RFLP method may not be accurate, the genotypic distribution was not departure from HWE in most studies (11 of 13 studies). However, the Taqman real-time PCR assay or other genotyping method may be needed to confirm the findings in a future study.

ASSOCIATIONS AND INTERACTIONS

Subjects with the low-activity genotype may be associated with decreased risk of lung cancer if EPHX1 enzyme acts as a mechanism for metabolic activation of several carcinogens present in tobacco smoke. Lower-activity *EPHX1* exon 3 genotypes have been associated with decreased lung cancer risk in several studies (Table 2; Appendix Table 3). A protective effect of low-activity genotype (His/His) of the *EPHX1* exon 3 was observed among blacks,[15] Spaniards,[18] and Austrians.[21] A French study also found that the *EPHX1* exon 3 His/His genotype was a protective factor for lung cancer.[11] Three studies of whites,[13,15,20] 1 Japanese study,[43] 1 black study[54] and 1 Mexican-American study[54] found no substantial relationships between the *EPHX1*exon 3 genotype and lung cancer risk. In contrast, 1 white study[1] and 2 Chinese studies[42,44] found that the low-activity genotype His/His was associated with a modest increase in risk of lung cancer.

The 11 case–control studies in 13 different ethnic populations of lung cancer and *EPHX1* genotype at exon 3 included 4075 subjects (1606 lung cancer cases and 2469 controls). The summary OR for the His/His (low-activity) genotype was 0.83 (0.61–1.12). The distribution of the *EPHX1* exon 3 genotypes among controls is not in agreement with HWE in 2 studies of whites.[1,20] A lack of equilibrium can indicate that the genotype distribution in the control group was not representative of the general population from which the cases presumably arose, suggesting the possibility of selection bias. In the study of Smith and Harrison, control selection was based on convenience sampling of blood donors.[1] In studies of whites with $P_{HWE} \geq 0.05$, the summary OR for the His/His genotype was 0.65 (0.44–0.96). On the other hand, the His/His genotype was only modestly associated with increased risk of lung cancer in Asians (1.37; 0.83–2.27). Evidence for heterogeneity and publication bias was absent in the analyses. A protective effect of the His/His genotype at exon 3, which is related to decreased EPHX1 activity, was suggested in whites. This finding could be explained by a predominant activating role of EPHX1 in the metabolism of lung carcinogens.

The Arg/Arg genotype at exon 4 polymorphism was weakly associated with increased risk of lung cancer among Chinese,[42] Mexican-Americans,[54] blacks,[54] and whites[20,21] (Table 3), and with decreased risk of lung cancer among Chinese[42] and whites.[18] The summary OR for the Arg/Arg (high-activity) genotype among the 11 case–control studies in 13 different ethnic populations (1625 lung cancer cases and 2486 controls) was 1.35 (0.94–1.92). In 7 white populations combined; the summary OR for the Arg/Arg genotype was 1.22 (0.79–1.90). In 2 Asian studies, the OR for lung cancer with the Arg/Arg genotype was 0.89 (0.20–3.90). Heteroge-

neity and publication bias were absent in the analyses. Thus, our metaanalysis indicates a weak promoting effect of the Arg/Arg genotype at exon 4 (which is related to increased EPHX1 activity) among whites. This finding could be also explained by a predominant activating role of EPHX1 in the metabolism of lung carcinogens.

When polymorphisms of *EPHX1* exon 3 and *EPHX1* exon 4 were combined, the predicted low activity was associated with decreased risk of lung cancer among 2 white populations shown in Table 4.[11,13] Lung cancer risk with these low-activity alleles was lower as predicted among one black[15] and 2 white studies.[18,20] However, studies among 2 white populations[1,32] and 1 Asian population[67] did not confirm the association. In a large American study, no relationship between the predicted low activity and lung cancer risk was found.[68] The 8 case–control studies of lung cancer and predicted EPHX1 activity in 9 different ethnic populations included 4614 subjects (2670 controls and 1944 lung cancer cases). The relations between various combinations of predicted EPHX1 activity showed a reduced risk with decreasing predicted activity. The summary OR for predicted low activity versus predicted high activity was 0.75 (0.53–1.07). The summary OR for predicted low activity versus predicted intermediate and high activities combined (including the study of Lin et al[75]) was 0.78 (0.58–1.04) (data not shown). The summary OR of predicted intermediate activity versus predicted high activity was 0.82 (0.61–1.09) (data not shown). Our results were robust in sensitivity analyses that were restricted to studies that were composed mostly of whites with $P_{HWE} \geq 0.05$ (0.76; 0.51–1.15) or studies of white only with $P_{HWE} \geq 0.05$ (0.72; 0.43–1.22). The Cochrane Q test for heterogeneity showed a statistical significance in both sensitivity analyses ($P = 0.004$ for mostly whites and $P = 0.003$ for all whites).

The presence of heterogeneity may compromise the interpretation of metaanalyses and result in erroneous and potentially misleading conclusions.[69,70] The presence of significant heterogeneity suggests that means the estimated OR in each study is not homogeneous and the estimated ORs are close to 1.0 in the larger studies. In fact, the largest study by Zhou et al showed no effect of *EPHX1* on lung cancer risk (OR = 0.99).[68] Possible sources of heterogeneity are ethnicity (the prevalence of the "at-risk" allele, ethnic differences in roles of the polymorphism), study design, and so on. Another possible reason for heterogeneity is linkage disequilibrium with additional allelic variants of *EPHX1* gene that modulate overall enzyme activity. Furthermore, it is possible that interaction with polymorphisms at other genes may be important. Heterogeneity can be taken into account by applying the random-effects model, however. The Begg's and Egger's tests for publication bias were not statistically significant in both analyses. The summary ORs suggest that the predicted low EPHX1 activity was related to decreased risk of lung cancer. Again, this result could be partly explained by its increased capacity to activate blood pressure and other polycyclic aromatic hydrocarbons.

Histologic data were available for 6 studies. For the relationship between the development of certain histologic types of lung cancer and the *EPHX1* exon 3 polymorphism, the OR for adenocarcinoma for the His/His genotype calculated relative to the Try/Try genotype was 0.40 (0.17–0.94), whereas squamous cell carcinoma and small cell carcinoma revealed no clear association among whites.[21] Lee et al performed a pooled analysis using a part of genetic susceptibility to environmental carcinogen data (included 4 published studies[11,15,18,42] and 4 unpublished studies), and also reported that the decreased OR for the His/His genotype at exon 3 was present for adenocarcinoma (0.45; 0.26–0.79) and squamous cell carcinoma (0.77; 0.49–1.19).[60] On the other hand, there was a positive association for squamous cell carcinoma (OR for the Try/His and His/His genotypes combined vs the Try/Try genotype = 3.23; 1.00–10.38) but not for adenocarcinoma.[44] As for exon 4 polymorphism, a modest increased risk for the Arg/Arg (high-activity) genotype was seen among small cell carcinoma cases (1.46; 0.62–3.41).[60] For predicted EPHX1 activity, there was suggestion of a slight increased risk for predicted high enzyme activity for adenocarcinoma (2.65; 0.97–7.21) and for squamous cell carcinoma (1.93; 0.62–5.95) among whites.[13] Among blacks with adenocarcinoma, there was a suggestion of increased risk with increasing predicted activity (OR for high activity = 1.86; 0.87–3.99).[15] Among Taiwanese, the ORs for predicted high/normal enzyme activity for squamous cell carcinoma and adenocarcinoma were 1.96 (1.04–3.70) and 0.65 (0.36–1.16), respectively.[67] There was a modestly increased OR for adenocarcinoma with predicted high activity (1.39; 0.95–2.05), but not for squamous cell carcinoma and small cell carcinoma.[60] In contrast, there was a suggestion of decreased risk with increasing predicted activity (OR for high activity = 0.38; 0.15–0.98) among whites with either squamous or small cell carcinoma.[15] No associations were seen between predicted EPHX1 activity and any histologic types of lung cancer in a study of whites.[68]

Taken together, results for the high-activity genotype or predicted high activity from combinations of exon 3 and exon 4 *EPHX1* genotypes and risk for different histologic types of lung cancer are conflicting and suggest that the genetically determined activity of EPHX1 in human tissues may not be completely predicted from these data. It is also possible that confounders that have not been controlled for may have interfered with the analysis.

INTERACTIONS

Gene–Environment Interactions

The gene–environment interactions explored discussed in the literature concerned features of cigarette smoking and genotype. Eight studies investigated interactions between cigarette smoking and *EPHX1* in relation to lung cancer. There was a strong association between *EPHX1* exon 3 genotypes and lung cancer risk among smokers (5.66; 1.71–18.68) but not in nonsmokers (0.66; 0.23–1.87).[44] In contrast, there was no clear modification of cigarette smoking according to *EPHX1* exon 3 polymorphism.[20,60] There was also no interaction between cigarette smoking and *EPHX1* exon 4 polymorphism.[20,60] A large American study, with significant interaction ($P < 0.01$) between predicted EPHX1 enzyme activity

TABLE 4. Studies of Predicted EPHX1 Activity and Risk of Lung Cancer

Citation	Race/Ethnicity	No. Cases	No. Controls	Predicted Low-Activity Genotype			Classification*
				Cases; %	Controls; %	Adjusted OR (95% CI)	
Smith et al,[1] UK	White	50	203	10.0	5.4	2.03 (0.49–7.39)†	S
Benhamou et al,[11] France	White	150	172	33.3	49.4	0.38 (0.19–0.75)	B
Lodon et al,[15] USA	Black	155	242	16.8	22.3	0.69 (0.36–1.30)	B
	White	182	458	36.8	36.0	1.56 (0.87–2.86)	B
Lin et al,[67] Taiwan	Taiwanese	132	259	59.1‡	61.0‡	1.03 (0.66–1.61)	S
To-Figueras et al,[18] Spain	White	175	187	31.4	37.4	0.68 (0.41–1.15)	B
Zhou et al,[68] USA	White (over 95%)	974	1142	14.4	13.7	0.86 (0.0.60–1.22)	S
Zhao et al,[20] USA	White	148	147	29.1	32.0	0.58 (0.30–1.11)	B
Cajas-Salazar et al,[13] Austria	White	110	119	27.3	28.6	0.41 (0.18–0.94)	B

Summary§	No. Populations	No. Cases	No. Controls	Frequency (%) of Predicted Low Activity (base on random-effects model)	OR (95% CI) for Predicted Low Activity (compared with predicted high activity)		Cochrane Q Test for Heterogeneity
					Random-Effects Model	Fixed-Effects Model	
All studies	8	1944	2670	27.8 (18.1–37.5)	0.75 (0.52–1.07)	0.82 (0.68–0.99)	0.004
White studies (mostly composed of whites)	7	1789	2428	28.7 (17.6–39.7)	0.76 (0.51–1.15)	0.84 (0.69–1.03)	0.003
White studies	6	815	1286	28.1 (16.1–40.2)	0.72 (0.43–1.22)	0.73 (0.56–0.96)	0.003

*Classification based on Smith and Harrison (S) or Benhamou et al (B).
†Crude OR.
‡Frequency of the predicted low and intermediate enzyme activity combined.
§Exclude the study by Lin et al because the predicted low enzyme activity was combined with the predicted intermediate activity.

and cigarette smoking, indicated that cumulative cigarette smoking played a pivotal role in the association between the *EPHX1* polymorphisms and lung cancer risk.[68] Smoking altered the direction of risk from 0.45 (0.22–0.93) in heavy smokers to 1.59 (0.80–3.14) in nonsmokers.[68] However, To-Figueras et al reported that the ORs for predicted high EPHX1 activity versus predicted low EPHX1 activity were 1.43 (0.66–3.11) among heavy smoker and 1.42 (0.70–2.87) among medium/light smokers.[18] Thus, their study found no interaction between predicted EPHX1 activity and cigarette smoking. There was also no appreciable difference in the association between predicted EPHX1 activity and lung cancer risk according to smoking status.[11,15,20,60,67]

It has been suggested that genetic polymorphisms may affect cancer risk, particularly at low carcinogen doses.[71] This could happen, for example, if the relevant enzyme is saturated in both low and high metabolizers at high-dose levels but not at low dose levels. If this is the case, it may not be apparent if all current smokers are grouped together. Broad categorization of tobacco exposure may prevent researchers from identifying genetically susceptible individuals who may have increased risk at low exposure levels. Significant interactions can be seen when tobacco exposure is divided into finer groups. Furthermore, Hassett et al reported that genotype and smoking information might be insufficient to explain the variation in EPHX1 enzyme activity.[72] Dietary factors such as fish oil may induce EPHX1 and thus increase enzyme activity,[73] and such phenotypic determinants may vary across populations. Given the possibility of environmental effects on EPHX1 activity, further work on interactions between *EPHX1* polymorphisms and smoking is needed.

Gene–Gene Interactions

Interaction with polymorphisms at other genes may also be important. Combined with CYP1A1, it has been reported that EPHX1 can metabolize polycyclic aromatic hydrocarbons into highly mutagenic and carcinogenic diol epoxides.[74,75] Lin et al found that a combination of the susceptible C/C genotype of *CYP1A1* T3801C polymorphism and predicted high EPHX1 enzyme activity was strongly associated with lung cancer (6.76; 2.29–19.1) compared with predicted high EPHX1 enzyme activity alone (1.96; 1.04–3.70) in patients with squamous cell carcinoma.[67] However, neither the *CYP1A1* T3801C nor the *CYP1A1* A2455G (Ile462Val) genotypes modified the association between predicted EPHX1 activity and lung cancer risk.[11] A significant interaction was found between the *EPHX1* Try113His polymorphism at exon 3 and *GSTP1* Ile105Val polymorphism at exon 5.[18] If only subjects with the Ile/Ile genotype of *GSTP1* were considered, an increased risk was associated with Try/Try of *EPHX1* (2.19; 1.12–4.28). However, considering only the subjects with one or 2 Val alleles of *GSTP1*, no risk was associated with the Try/Try genotype of *EPHX1* (0.89; 0.45–1.77). No interaction has been found between *EPHX1* and *GSTM1* genes[11,15,18] or between *EPHX1* and *GSTT1*.[18] The results of gene–gene interactions are limited to few studies with small sample size, and so they may not provide reliable information. In addition to adequate sample size, assessment of gene–gene interaction also depends on the proper statistical evaluation of interaction with multiplicative and additive models.

LABORATORY TESTING

Methods of genotyping for the exon 3 polymorphism of *EPHX1*[26] and the exon 4 polymorphism of *EPHX1*[10] by means of the polymerase chain reaction and restriction fragment length polymorphism techniques have been described previously.

POPULATION TESTING

To date, there is insufficient evidence implicating *EPHX1* in the etiology of lung cancer to consider population testing.

OTHER POTENTIAL PUBLIC HEALTH APPLICATIONS

At this writing, the available data are insufficient to support any public health recommendations.

DISCUSSION AND RECOMMENDATIONS FOR RESEARCH

In our metaanalyses, the low-activity genotype at exon 3 was associated with a 35% decrease in lung cancer risk among whites (Table 2). However, the polymorphism at exon 4 and the predicated EPHX1 activity were not associated with lung cancer risk among whites (Tables 3 and 4), however. Our results are consistent with the pooled analysis by Lee et al[69] In their pooled analysis, a 30% decease in lung cancer risk was observed for the His/His genotype at exon 3 (0.70; 0.51–0.96), whereas no effect for the exon 4 polymorphism was detectable. Because the polymorphism at exon 4 has been identified within the coding region of the gene, the substitution may be more likely the result of altered protein stability and not enzyme-specific activity. The molecular basis for variation in EPHX1 activity may not be characterized completely. There are conflicting reports on the association between both *EPHX1* polymorphisms and lung cancer risk in different populations, although there have been only a few studies among populations other than whites. Although the reasons for the inconsistencies across studies are not clear, small sample size may be a problem. Another possibility is that ethnic differences may reflect different gene–gene interactions or different linkages to the polymorphisms determining lung cancer risk.

Although the summary risk for developing lung cancer in individuals at each genotype may not be large, lung cancer is such a common malignancy that even a small increase in risk can translate to a large number of excess lung cancer cases. Therefore, polymorphisms, even those not strongly associated with lung cancer, should be considered as a potentially important public health issue. In addition, a susceptibility factor in one population may not be a factor in another. There are differences in the prevalence of *EPHX1* polymorphisms (as well as *CYP1A1*, *CYP2D6*, *CYP2E1*, *NAT2*, *GSTM1*, *GSTT1,* and *GSTP*)[76,77] across populations (Appendix Tables 1 and 2). In a population in which the prevalence of an

"at-risk" genotype in a given polymorphism is very low, the "at-risk" allele or "at-risk" genotype may be too infrequent to assess its associated risk. At a population level, the attributable risk must be small simply because it is an infrequent allele.

Research into the role of *EPHX1* polymorphisms in lung cancer is still in its early stages. As suggested by IARC and Todd, priorities for studies on molecular epidemiology should include large sample size, an independent replication followed by an initial study, biologic plausibility and physiologically, meaningful data supporting the functional role of the polymorphism in question.[78–80] The initial studies showed substantial variations in risk of developing lung cancer in individuals with specific genotypes. Even so, etiology of lung cancer cannot be explained by allelic variability at a single locus. Advances in identification of new variants and in high-throughput genotyping techniques will facilitate analysis of multiple polymorphisms within the genes along the same pathway.[81] Therefore, it is likely that definitive studies in the future will require analysis of large samples of cases and controls.[82,83]

The major burden of lung cancer in the population probably results from complex interaction between many genetic and environmental factors over time. The effects of polymorphisms are best represented by their haplotypes. Recently developed haplotype-based methods were not used in the studies we reviewed; however, it can be anticipated that in future association studies on lung cancer, the development of new approaches will include evaluation of haplotypic effects, either for selected polymorphisms physically close to each other or for multiple genes within the same drug-metabolism pathway.

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